

# Intravascular haemolysis and thrombocytopenia in left ventricular outflow obstruction<sup>1</sup>

Robert J. Jacobson, Charles E. Rath, and Joseph K. Perloff<sup>2</sup>

*From the Department of Medicine, Georgetown University School of Medicine, Divisions of Cardiology and Hematology, Georgetown University Hospital, Washington D.C., U.S.A.*

*Twenty-four patients with various types of isolated discrete aortic stenosis of a wide range of severity or with muscular obstruction to left ventricular outflow were studied haematologically and by cardiac catheterization and angiocardiology. Patients had haematocrits, reticulocyte, platelet, and megathrombocyte counts, serum haptoglobin, and lactic dehydrogenase performed; urine specimens were examined for haemosiderin and peripheral blood smears examined for schistocytes. Seventeen patients had <sup>51</sup>chromium red blood cell survival tests and 7 patients had plasma heme pigment determinations.*

*In discrete aortic stenosis (supravalvular, valvular, or subvalvular), intravascular haemolysis developed when pressure gradients of 50 mmHg or more were achieved. Gradients of this magnitude caused damaging shear stresses of at least 4000 dynes/cm<sup>2</sup> on red blood cells. Intravascular haemolysis was also found in 2 patients with functionally normal bicuspid aortic valves. In idiopathic hypertrophic subaortic stenosis, intravascular haemolysis was found in 4 of 7 patients and appeared to be related to the presence of a left ventricular-aortic systolic gradient and/or the rapid rate of ejection. Of the 24 patients, 10 had an increased megathrombocyte count; 5 of these had thrombocytopenia. Four patients with thrombocytopenia also had intravascular haemolysis, but there was no clear correlation between increased peripheral destruction of platelets and the haemodynamic findings.*

Despite a lively interest in intravascular haemolysis in patients with prosthetic valves or acquired valvular heart disease (Eyster, Mayer, and McKenzie, 1968; Myhre and Dale, 1971; Sears and Crosby, 1965; Wallace, Kenep, and Blakemore, 1970), no studies have dealt specifically with red blood cell and platelet survival in isolated obstruction to left ventricular outflow of broad ranges of severity and various morphological types. In our study, evidence of intravascular haemolysis and decreased platelet survival was sought in 24 patients with valvular, supravalvular, discrete subvalvular, or muscular subaortic stenosis, in whom severity ranged from functionally normal bicuspid aortic valves to severe obstruction.

## Subjects and methods

Studies were performed on 22 patients from Georgetown

University Hospital and two from the Cardiology Branch of the National Heart and Lung Institute, Bethesda, Maryland. The cardiac diagnoses were: non-obstructive, i.e. functionally normal, bicuspid aortic valves (2 patients); supravalvular aortic stenosis (1 patient); valvular aortic stenosis (12 patients); discrete subvalvular aortic stenosis (1 patient); idiopathic hypertrophic subaortic stenosis (7 patients); and 1 patient with focal calcific deposits in an aortic valve in which neither stenosis nor regurgitation could be shown at rest, during exercise, isoprenaline infusion, or Valsalva's manoeuvre. It was not determined whether the valve was bicuspid or tricuspid.

All 24 patients had retrograde or transseptal left heart catheterization with aortic root cineangiography. The haematological studies were done either before cardiac catheterization or at least one month after the procedure. All patients had haematocrit volumes performed by the microcapillary method, reticulocyte counts, and platelet counts determined by phase microscopy (Brecher and Cronkite, 1950).

Peripheral blood smears were prepared with Wright-Giemsa stain and were examined for red blood cell fragments or schistocytes, poikilocytes, burr, and helmet cells. Megathrombocyte counts were also obtained by

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<sup>2</sup> Present address: Section of Cardiology, Hospital of the University of Pennsylvania, 3400 Spruce Street, Philadelphia, Pennsylvania 19104, U.S.A.

examining the peripheral blood smears with the use of a calibrated ocular micrometer. The method used was similar to that of Garg, Amorosi, and Karpatkin (1971), and platelets with a diameter greater than  $2.5 \mu$  were registered as megathrombocytes. In 8 patients bone marrow aspirations were performed and bone marrow smears, prepared with Wright-Giemsa stain, were examined. Iron stores were assessed using ferrocyanide (Prussian blue) stain.

Serum haptoglobin was determined by the method of Javid and Horowitz (1960). Serum lactic dehydrogenase was done by the method of Wacker, Ulmer, and Vallee (1956), and the upper limit of normal in our laboratory is 120 units. Plasma heme pigments were measured by the method of Crosby and Furth (1956), (normal 1 to 4 mg/100 ml). Coombs tests with a broad spectrum anti-serum, haemoglobin electrophoresis using cellulose acetate strips, glucose-6-phosphate dehydrogenase (G6PD) deficiency screening tests (Beutler, 1966), and osmotic fragility tests were performed to exclude other causes of haemolysis. Urine specimens were obtained from the 24 patients during periods of activity, and haemosiderin in the urinary sediments was determined by Prussian blue staining.

Erythrocyte survival was studied in 17 patients using  $^{51}\text{Cr}$  tagged red blood cells according to the method of Wagner (1968). The red cell radioactivity was plotted against time on semilogarithmic co-ordinates. Normal values at this hospital for the half-life of red blood cells are 25 to 35 days. Using Wagner's method (1968), sequestration of the red blood cells was measured in two patients during the  $^{51}\text{Cr}$  red cell survival study.

## Results

Haemolysis was diagnosed when the  $^{51}\text{Cr}$  red blood cell (RBC) survival test was 24 days or less or the plasma heme pigments were increased and serum haptoglobin was decreased. Anaemia was considered present when the haematocrit was less than 40 per cent in men or less than 37 per cent in women. Of the 24 patients studied, 15 showed evidence of intravascular haemolysis. Eleven of the 15 patients had compensated haemolytic states and 4 patients had mild haemolytic anaemia with haematocrits ranging from 32 to 38 per cent. The Coombs test was negative in all 24 patients.

In three patients (Cases 11, 17, and 20), a decreased  $^{51}\text{Cr}$  survival test was the only evidence of haemolysis. The serum lactic dehydrogenase was raised in 3 of the 15 patients with haemolysis and correlated poorly with other evidence of haemolysis. Splenic sequestration studies performed in 2 patients with decreased erythrocyte survival, did not reveal hypersplenism. Bone-marrow aspirates were performed on 2 patients without and 6 with intravascular haemolysis. Moderate to pronounced erythroid hyperplasia was found in all the patients with haemolysis and 2 of these patients (Cases 7 and 18) had decreased iron stores.

The haemodynamic findings in the patients with discrete aortic stenosis or with functionally normal bicuspid or focally calcified aortic valves are shown in Table 1. The haematological findings in these

TABLE 1 *Haemodynamic findings in 17 patients without idiopathic hypertrophic subaortic stenosis*

Case No.	Sex	Age (yr)	Diagnosis	Peak systolic pressure gradient	Orifice size	Cardiac index	Other cardiac findings
<i>Without haemolysis</i>							
1	M	47	Functionally normal aortic valve	None	—	3.4	Focal valvular calcification
2	M	8	Supravalvular aortic stenosis	44	—	3.9	Mild peripheral pulmonary artery stenosis
3	M	52	Valvular aortic stenosis	45	0.6	2.7	Congestive heart failure
4	M	26	Valvular aortic stenosis	37	1.3	3.1	Valve calcified
5	F	46	Valvular aortic stenosis	47	0.8	2.9	Valve calcified
6	M	61	Valvular aortic stenosis	54	0.5	2.0	Valve calcified; congestive heart failure
<i>With haemolysis</i>							
7	M	36	Valvular aortic stenosis	80	0.5	2.6	Valve calcified
8	M	49	Valvular aortic stenosis	68	0.6	2.9	Mild aortic incompetence
9	M	48	Valvular aortic stenosis	115	0.7	3.8	Valve calcified
10	M	57	Valvular aortic stenosis	70	0.7	3.3	Valve calcified
11	M	36	Valvular aortic stenosis	75	0.9	3.2	—
12	F	61	Valvular aortic stenosis	140	—	—	—
13	F	76	Valvular aortic stenosis	52	0.5	2.3	Valve calcified
14	F	64	Valvular aortic stenosis	84	—	—	Valve calcified
15	F	33	Fixed subvalvular aortic stenosis	55	0.8	3.0	Mild aortic incompetence
16	M	10	Bicuspid aortic valve	None	—	3.5	—
17	M	52	Bicuspid aortic valve	None	—	—	Mild aortic incompetence

TABLE 2 *Haematological findings in the 17 patients without idiopathic hypertrophic subaortic stenosis*

Case No.	Diagnosis	Haematocrit (%)	Reticulocytes (%)	<sup>51</sup> Cr RBC survival (dy)	Haptoglobin (mg/100 ml)	Heme pigment (mg/100 ml)	LDH	Blood smear schistocytes	Platelet count (1000/mm <sup>3</sup> )	Mega-thrombocytes (%)
<i>Without haemolysis</i>										
1	Functionally normal aortic valve	43	1.7	—	180	Normal	97	Normal	183	Normal
2	Supravalvular aortic stenosis	36	2.2	—	60	2.0	138	Normal	316	Normal
3	Valvular aortic stenosis	41	0.2	35	102	—	—	Normal	210	Normal
4	Valvular aortic stenosis	40	1.3	25.5	121	—	—	Normal	261	Normal
5	Valvular aortic stenosis	39	2.4	30	137	—	95	Normal	410	Normal
6	Valvular aortic stenosis	49	2.0	—	99	2.0	—	Normal	245	50
<i>With haemolysis</i>										
7	Valvular aortic stenosis	38	2.7	—	0*	—	32	10/1000 RBC	116	33
8	Valvular aortic stenosis	40	1.7	14	6	—	130	17/1000 RBC	140	18
9	Valvular aortic stenosis	48	2.3	—	22	9.0	86	Normal	282	Normal
10	Valvular aortic stenosis	40	3.1	17.5	18	21.0	104	Normal	167	Normal
11	Valvular aortic stenosis	40	1.5	22	119	—	—	Normal	261	20
12	Valvular aortic stenosis	40	0.9	16	18	—	—	Normal	255	Normal
13	Valvular aortic stenosis	37	4.0	18.5	85	—	86	Normal	255	Normal
14	Valvular aortic stenosis	36	1.8	17.5	—	—	76	10/1000 RBC	99	23
15	Fixed subvalvular aortic stenosis	38	2.2	—	23	45.0	104	Normal	250	Normal
16	Bicuspid aortic valve	32	1.5	15.3	42	7.5	75	30/1000 RBC	320	Normal
17	Bicuspid aortic valve	49	1.5	18.5	126	—	72	10/1000 RBC	203	41.5

\* Haemosiderinuria.

patients are shown in Table 2. Of 12 patients with valvular aortic stenosis, there were 8 with intravascular haemolysis and 2 of these (Cases 7 and 14) were mildly anaemic. Case 7 also had complete absence of haptoglobin and haemosiderinuria.

In the Fig., the peak systolic pressure gradients in the 14 patients with supravalvular and valvular aortic stenosis and isolated discrete subvalvular aortic stenosis are shown on the left. The 5 patients without intravascular haemolysis had pressure gradients less than 55 mmHg, whereas the 9 patients with intravascular haemolysis had pressure gradients ranging from 50 to 140 mmHg. Two patients (Cases 3 and 6) with peak systolic pressure gradients of 45 and 54 mmHg, respectively (open circles in Fig.), were in congestive heart failure. They had severely

stenosed aortic valves but no intravascular haemolysis.

Both patients with functionally normal, non-stenotic bicuspid aortic valves had intravascular haemolysis. Case 16, a boy of 12 years had a haematocrit of 32 per cent with normochromic, normocytic erythrocytes, and a <sup>51</sup>Cr red cell survival of 15 days. The other patient, Case 17, a 52-year-old man, had a <sup>51</sup>Cr red cell survival test of 18.5 days but no anaemia. His angiocardigraphic studies revealed trivial aortic regurgitation associated with a non-stenotic bicuspid valve.

Of the 7 patients with idiopathic hypertrophic subaortic stenosis, 4 had evidence of intravascular haemolysis (Table 3). One patient, Case 18, was anaemic with a haematocrit of 33.5 per cent, reticu-

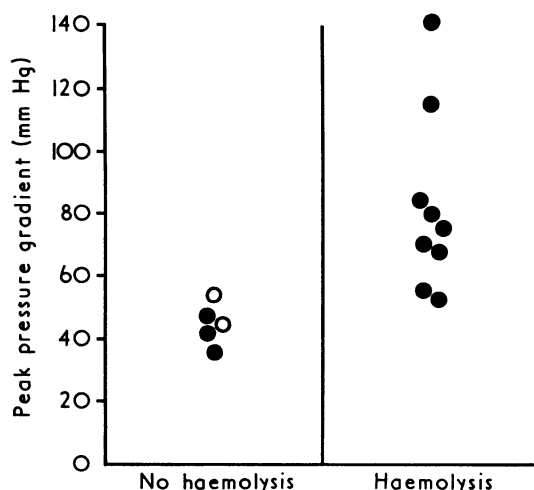


FIG. The relation between the peak systolic pressure gradient and intravascular haemolysis in 14 patients with discrete aortic stenosis. Open circles indicate patients in congestive heart failure.

locyte count of 4 per cent, and a peripheral blood smear that revealed schistocytes, burr, and helmet cells. The osmotic fragility study of her erythrocytes was normal. All the patients with idiopathic hypertrophic subaortic stenosis had normal haemoglobin electrophoreses and were not G6PD deficient. Systolic pressure gradients were present in Cases 18 and 19 at rest. In the other 5, pressure gradients of varying degrees could be elicited by physical or pharmacological intervention, e.g. Valsalva manoeuvre, isoprenaline infusion, or amyl nitrite inhalation (Table 3).

Of the 24 patients, 10 had an increased megathrombocyte count (i.e. greater than 16%) and 5 of these 10 had thrombocytopenia. None of the patients in whom a bone-marrow aspiration was performed had decreased numbers of megakaryocytes. Both intravascular haemolysis and thrombocytopenia were found in 4 of the 24 patients.

### Discussion

Intravascular haemolysis has been identified in a number of disorders affecting the heart and great vessels. Haemolysis has been reported in patients with prosthetic heart valves (Sears and Crosby, 1965; Eyster, Rothchild, and Mychajliw, 1971), aortic and mitral valve disease (especially aortic stenosis and aortic and mitral regurgitation) (Eyster *et al.*, 1968; Myhre and Dale, 1971; Brodeur *et al.*, 1965a), ruptured sinus of Valsalva aneurysm (Ellman and Knox-Macauley, 1970), endocardial cushion defects repaired with 'Teflon' patches (Sigler *et al.*, 1963), and coarctation of the aorta (Ravenel, Johnson, and Sigler, 1969). Recently Harker and Slichter (1970) reported decreased platelet survival in patients with prosthetic valves.

In subjects with acquired valvular disease the mechanism for intravascular haemolysis is believed to relate to turbulence and shear stress produced by flow through stenosed or incompetent orifices (Dameshek and Roth, 1964; Brodeur *et al.*, 1965b). Shear stress is the force imposed per unit area of interface between two fluid layers sliding past each other (LaCelle and Weed, 1971). When erythrocytes are subjected to shear stresses exceeding the limit of the tensile strength of their membranes, the membranes rupture or tear with the formation of

TABLE 3 Haemodynamic and haematological findings in 7 patients with idiopathic hypertrophic subaortic stenosis

Case No.	Age/Sex	Haematocrit (%)	Reticulocytes (%)	<sup>51</sup> Cr RBC survival (days)	Platelet count/mm <sup>3</sup>	Mega-thrombocyte (%)	Peak pressure gradient	
							Control	With intervention
With haemolysis								
18*	73/F	33.5	4.0	12.5	132,000	25.5	100	—
19	35/M	45.5	3.0	15	195,000	27.7	70-90	—
20	28/M	46	1.2	21	257,000	Normal	0	80
21	47/M	44	2.2	—†	200,000	Normal	0-5	120
Without haemolysis								
22	55/M	43.5	2.1	28	139,000	50	0-3	95
23	17/M	47.5	1.9	30	165,000	Normal	0	65
24	20/M	51	0.6	26	233,000	22.3	0	20

\* Blood smear schistocytes 70/1000 RBC, LDH 200 units.

† Plasma haemoglobin 12.0 mg/100 ml, LDH 260 units.

red blood cell fragments or schistocytes. In studies by Nevaril *et al.* (1968) the critical or threshold shearing stress for normal erythrocytes is 3000 dynes per  $\text{cm}^2$ , i.e. little haemolysis occurs at stresses below 3000 dynes/ $\text{cm}^2$ , but above this level haemolysis is greatly increased.

In discrete aortic stenosis, the obstruction to outflow imposes maximum shear stress on circulating erythrocytes as the turbulent jet stream is ejected through the narrowed orifice. The shear stress increases as the systolic pressure gradient increases. By the use of Bernoulli's equation<sup>1</sup> (Nevaril *et al.*, 1968), the shear stress exerted on red cells when the systolic pressure gradient is 50 mmHg, is calculated to be 4000 dynes/ $\text{cm}^2$ . In the present study, the 9 patients with valvular or discrete subvalvular aortic stenosis and pressure gradients greater than 50 mmHg were calculated to have shear stresses of 4000 dynes/ $\text{cm}^2$  or more, sufficient to damage normal red blood cells and cause intravascular haemolysis. Cases 3 and 6 had severely stenosed aortic valves and were in congestive heart failure. They had reduced pressure gradients and their erythrocytes were not subjected to critical shearing stresses and did not haemolyse.

It has been postulated (Edwards, 1961) that during systole the central zone of a non-stenotic bicuspid aortic valve is unobstructed but the peripheral zones near the commissures may not effectively open. Red cells could, therefore, be subjected to increased shearing stresses as adjacent layers of blood flow through the valve at different rates. Intravascular haemolysis in 2 of our patients with non-stenotic bicuspid aortic valves might be explained on this basis.

Idiopathic hypertrophic subaortic stenosis is characterized by a wide variability of left ventricular-aortic pressure gradients. A relation may exist between the presence of a gradient, rapid ejection, and intravascular haemolysis (Table 3). When Case 18 was treated with propranolol 160 mg/day, her haematocrit became normal, suggesting that with a reduction in gradient and/or rate of ejection, the shear stresses on the erythrocytes were lessened.

In disorders causing increased destruction of platelets, Garg *et al.* (1971) showed that thrombocytopenia was associated with increased megathrombocytes in the peripheral blood and increased megakaryocytes in the bone marrow. They also described a compensated thrombocytolytic state in which both megathrombocytes and megakaryocytes were increased but the platelet count remained normal. The finding of thrombocytopenia and increased

megathrombocytes in patients with left ventricular outflow obstruction may indicate increased peripheral destruction of platelets. Shear stress related to left ventricular outflow obstruction may play a role in causing the increased destruction of platelets, but in this study no direct correlation could be found between platelet abnormalities and the haemodynamic findings. Intravascular haemolysis can cause intravascular coagulation (Brain, 1969; Baker *et al.*, 1968); while we have no evidence that intravascular coagulation occurred in these 24 patients, 4 had the combination of both intravascular haemolysis and thrombocytopenia.

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<sup>1</sup>  $t = 0.03 \frac{2\Delta P}{e}$  dynes/ $\text{cm}^2$ ;  $t$  = shear stress;  $e$  = the density;  $\Delta P$  = pressure gradient.

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Requests for reprints to Dr. Robert J. Jacobson, Georgetown University Hospital, Department of Medicine, 3800 Reservoir Road, Washington D.C. 20007, U.S.A.